Supplementary Information

Transformation Products and Human Metabolites of Triclocarban and Triclosan in Sewage Sludge across the United States

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Number of Figures: 1

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List of Acronyms

2'-OH-TCC	2-Hydroxy-Triclocarban (a human TCC metabolite)
2'-SO_3-TCC	2'-Sulfonate-Triclocarban
3'-Cl-TCC	3'-Chloro-Triclocarban (a manufacturing byproduct of TCC)
3'-OH-TCC	3'-Hydroxy-Triclocarban (a human TCC metabolite)
3-CA	3-Chloroaniline
4-CA	4-Chloroaniline
ACN	Acetonitrile
AD	Anaerobic Digestion/Digester
B#	Biosolids from site #
DCC	Dichlorocarbanilide (a dechlorination product and manufacturing byproduct of TCC)
EPA	Environmental Protection Agency
GC	Gas Chromatography
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MCC	Monochlorocarbanilide (a dechlorination product of TCC)
MeTCS	Methyl-Triclosan (a microbial metabolite of TCS)
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NCC	Carbanilide (an industrial chemical and dechlorination product of TCC)
NIH	National Institutes of Health
NIEHS	National Institute of Environmental Health Sciences
S#	Sewage sludge from site #
TCC	Triclocarban
TCS	Triclosan
U.S.	United States
USGS	United States Geological Survey
WWTP	Wastewater Treatment Plant

Supplementary Descriptions

Solvent Extraction. Sewage sludge and biosolids samples were shipped between labs on dry ice and stored at -80ºC until thawed for extraction and analysis at Arizona State University. Triplicate samples aliquots were prepared in glass vials as wet samples (approximately 0.5 g) and dried at 70°C in a stationary incubator until their mass stabilized. The analytes of interest were extracted with 50:50 methanol:acetone solution containing 10 mM acetic acid by sonication for 4 h. The biosolids particles were physically ground to finer particulates while suspended in the organic solvent prior to sonication to increase the surface area exposed to the solvent. The particulates were allowed to settle overnight, after which the organic extract was transferred to a new vial and evaporated to dryness at 37°C using a gentle stream of nitrogen in a ReactiVap Evaporator (Thermo Scientific). The samples were reconstituted using 4 mL methanol and placed in a sonication bath until all debris were dissolved or suspended. The extracts were filtered using a 0.2 μm polytetrafluoroethylene membrane prior to analysis. All added volumes and extract masses were gravimetrically determined (instead of relying on the precision and accuracy of the micropipette) and tracked during the extraction procedure, respectively, using an analytical balance (Mettler-Toledo) to minimize intersample variability. The averaged wet and dry weights of the extracted samples were 0.777 ± 0.164 g and 0.138 ± 0.027 g, respectively. All extracts were stored at -20°C prior to analysis. The concentrations provided here are all on a dry weight (dw) basis.

LC-MS/MS Analysis. Prior to analysis, the extracts were fortified with 5 ng ^{13}C-labeled standards for TCC and TCS. The samples were diluted in a 2:1 methanol/water mixture of which 100 µL was injected. The LC was run at 0.4 mL/min starting at 60% acetonitrile (ACN) with a ramp to 90% ACN over 7 min, held at 90% ACN for 2 min, decreased back to 60% ACN over 1 min, and held at 60% ACN for 3 min. The analytes of interest were separated on an X-Bridge 4.6 × 150 mm C8 column with 3.5 μm particle size (Waters) preceded by an equivalent guard column

using a gradient LC protocol on a Prominence instrument (Shimadzu) and determined using an API 4000 triple quadrupole mass spectrometer (MS/MS, ABSciex). A switching valve allowed sample to flow to the MS/MS between 4 and 12 min of each 13 min run. The source parameters were set as follows: curtain gas = 25 psi, gas 1 = 70 psi, gas 2 = 50 psi, Ion Source = -4500 eV, temperature = 500ºC, entrance potential (EP) = -10 eV, and collision activated dissociation (CAD) gas = 12 psi.

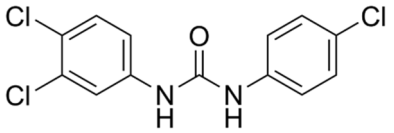
GC-MS/MS Analysis. Biosolids extracts were prepared for GC-MS/MS analysis by diluting the extract in methanol to 80% with the addition of the ^{13}C-labeled standard. An Agilent 7890 GC with an Agilent 7000-series tandem mass spectrometer was used for the quantification of MeTCS. The column used was an Agilent HP-5ms, 30 m x 250 μm x 0.25 μm. An injection volume of 1 μL was introduced to a split/splitless inlet in splitless mode. The inlet temperature was isothermal at 275ºC. The oven temperature program started at 150ºC with a 10ºC/min ramp to 250ºC, followed by a 5-min hold at 300ºC. The carrier gas was helium, supplied at a rate of 1.2 mL/min. The mass spectrometer utilized electron impact ionization with an ionization energy of 70 eV. The mass spectrometer gain was set to 100.

Quality Assurance/Quality Control. All analytes and their respective labeled standards were identified using their specific retention time and two multiple reaction monitoring transitions (Table S2 and S3). Reagent blanks were processed and extracted alongside sludge samples and all analyses included solvent blanks, standards solutions, and instrument performance standards. All reported concentrations were determined based on a linear fit to data from 5-8 calibration standards with $R^2 \geq 0.999$ for all analytes. Method detection limits were not determined due to a lack of pristine biosolids reference material according to USGS guidelines.[48] Instead instrumental limits of detection (LOD) and quantification (LOQ) were determined according to the EPA guidelines described in 40 CFR 136, Appendix B.[55] Briefly, the LOD and LOQ were calculated by multiplying the standard deviation on the calculated concentrations of eight replicate injections

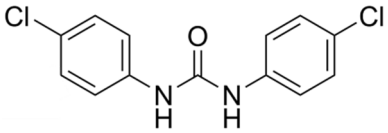
of low concentration standards with a factor 3 and 10, respectively. The dynamic ranges, LODs, and LOQs, provided in Tables S4 and S5, were determined from solvent standards and these ranges and limits were applied to solvent extract concentrations. The recoveries presented in Tables S4 and S5 were determined by spiking triplicate biosolids samples with two levels of a known mass of the target analytes, and by determining the recovery of the mass differential using triplicate, unspiked biosolids samples.

Statistical Analyses. All statistical analyses were performed using SPSS v 21 (IBM) unless another software package is mentioned explicitly. The moving window analysis was performed using a paired two-tailed Student *t*-test with $\alpha = 0.05$ and $\alpha = 0.01$ as thresholds for significance. Removal with different sludge treatment techniques (sludge heating, drying, and anaerobic digestion) and the inter-study comparison of TCC and TCS occurrence in biosolids were evaluated using a two-tailed Student *t*-test of two samples with equal variance. Correlation analyses for the different co-contaminants was performed using a linear model with non-forced intercept in Microsoft Excel Professional 2013 and there were no non-detects in the datasets.

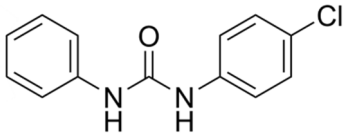
Supplementary Figures



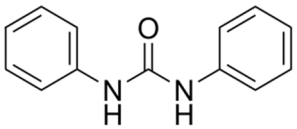
Triclocarban (TCC)



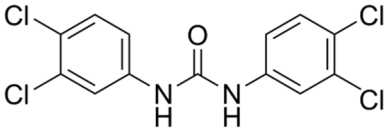
Dichlorocarbanilide (DCC)



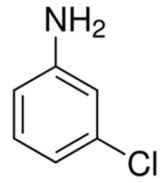
Monochlorocarbanilide (MCC)



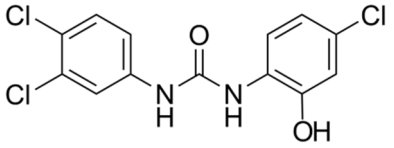
Carbanilide (NCC)



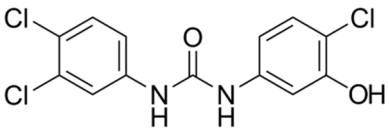
3'-Chloro-triclocarban (3'-Cl-TCC)



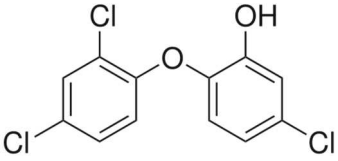
3-Chloroaniline (3-CA)



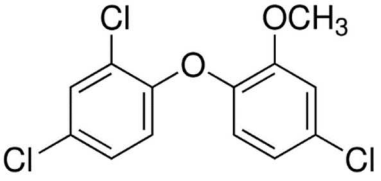
2'-Hydroxytriclocarban (2'-OH-TCC)



3'-Hydroxytriclocarban (3'-OH-TCC)



Triclosan (TCS)



Methyl triclosan (MeTCS)

Figure S1. Structural formulas of 10 targeted chemicals.

Supplementary Tables

Table S1. Facilities sampled for sewage sludge (S) and biosolids (B), including information about the size of the WWTPs feeding the 14 separate facilities, the number of dates that sludge or biosolids were collected and analyzed, and general characteristics of the biosolids treatment strategy; if land applied biosolids were either designated as Class A or B materials; biosolids not rated (NR) were not land applied.

Sample type/Plant ID	Dates Sampled (#)	WWTP Flow (MLD)	Biosolids Classification	Biosolids Treatment Approach
Biosolids (2009-2010)				
B4	1	>150	Class A	AD + storage + composting
B5	1	20-100	-	Two sequential aerobic digesters
B6-1	1	≤10	NR	AD
B6-2	1	≤10	NR	AD + dewatering
B7	2	>150	Class B	AD + aging
B8	2	20-100	Class A	AD + storage
B9	1	≤10	Class B	Extended storage
B10	1	≤10	Class B	AD
B11	1	≤10	NR	AD + composting
B12	1	20-100	NR	AD
B13	1	>150	Class B	AD
B14-1	2	>150	Class B	AD
B14-2	2	>150	Class A	AD + heat drying (pelletized)
B15	1	>150	Class B	AD
B16-1	23	>150	Class B	AD
B16-2	1	>150	Class A	AD + composting with green waste
Sewage Sludges				
S15	1	>150	NA	
S16	2	>150	NA	
S20	1	≤10	NA	settlement tank sludge

AD = Anaerobic Digestion; NA: Not applicable.

Table S2. Overview of the values for compound-specific LC and MS/MS parameters.

Analyte	Polarity	Transition (Confirmation Ion)	DP (V)	CE (V)	CXP (V)	RT (min)	DT (ms)
TCS	-	289>35 ($^{13}C_{12}$ TCS)	-60	-34	-3	10.01	50
$^{13}C_{12}$ TCS	-	301>35(NA)	-60	-34	-3	10.01	50
TCC	-	313>160($^{13}C_{13}$ TCC)	-80	-18	-9	9.54	50
$^{13}C_{13}$ TCC	-	326>166(NA)	-80	-18	-9	9.54	50
DCC	-	279>126(NA)	-70	-20	-11	8.62	50
MCC	-	245>126(NA)	-56	-16	-4	7.81	50
NCC	-	211>92(NA)	-60	-24	-3	7.03	50
2'-OH-TCC	-	329>168 (142)	-50 (-50)	-22 (-30)	-6 (-6)	9.06	50
3'-OH-TCC	-	329>168 (142)	-55 (-55)	-15 (-30)	-5 (-3)	7.81	50
3'-Cl-TCC	-	349>160 (162)	-60 (-60)	-30 (-25)	-5 (-5)	10.46	50
3-CA	+	128>93 (75)	61 (61)	27 (45)	8 (12)	6.85	100

DP = Declustering Potential; CE = Collision Energy; CXP = Collision Cell Exit Potential; RT = Retention Time; DT = Dwell Time; NA = Not applicable

Table S3. Overview of the values for compound-specific GC and MS/MS parameters.

Analyte	Transition (Confirmation Ion)	EI (V)	CID (V)	RT (min)	DT (ms)
MeTCS	302>252 (189)	70	30 (40)	8.90	100
$^{13}C_{13}$ MeTCS	314>264 (NA)	70	30 (NA)	8.90	100

EI = Electron Ionization; CID = Collision-Induced Dissociation; RT = Retention Time; DT = Dwell Time; NA = Not applicable

Table S4. Method performance characteristics for analytes quantified by LC-MS/MS and the results of the spike-recovery experiments.

Analyte	Calibration range (ng/L)	LOD (ng/L)	LOQ (ng/L)	0.1 µg/g dw spike-rec. (%)	1 µg/g dw spike-rec. (%)
***TCS**	30 – 35,000	35	117	NA	149 ± 63
***TCC**	10 – 35,000	4	13	NA	121 ± 28
DCC	10 – 3,500	3	9	89 ± 9	89 ± 1
MCC	10 – 3,500	32	107	101 ± 3	90 ± 2
NCC	10 – 3,500	3	9	109 ± 4	88 ± 1
2'-OH-TCC	10 – 35,000	72	241	108 ± 53	87 ± 2
3'-OH-TCC	10 – 35,000	45	149	111 ± 27	83 ± 2
3'-Cl-TCC	10 – 35,000	16	54	100 ± 4	90 ± 2
3-CA	30 – 35,000	26	86	58.9 ± 41.2	92.3 ± 12.1

LOD = Limit of Detection; LOQ = Limit of Quantification; rec. = Recovery; dw = dry weight

* Isotope-dilution mass spectrometry available

Table S5. Method performance characteristics for analytes quantified by GC-MS/MS and the results of the spike-recovery experiments.

Analyte	Dynamic range (ng/L)	LOD (ng/L)	LOQ (ng/L)	0.1 μg/g dw spike-rec. (%)	1 μg/g dw spike-rec. (%)
***MeTCS**	1,000 – 25,000	130	440	88 ± 30	110 ± 19

LOD = Limit of Detection; LOQ = Limit of Quantification; rec. = Recovery; dw = dry weight

* Isotope-dilution mass spectrometry available

Table S6. Results of the moving window analysis for changes in concentration of the chemical monitored and detected for 12 months at plant #16 (concentrations presented in Figure 1). The *p*-values were calculated with a paired two-tailed Student *t*-test using concentration data generated from a pair of triplicate biosolids samples. Significant changes in concentration ($\alpha = 0.05$) are presented in bold and the number of significantly different concentrations changes is presented below for two thresholds ($\alpha = 0.05$ and $\alpha = 0.01$).

Time Period (starting 3/15/2009)	***p*-values**							
	TCC	**DCC**	**MCC**	**2'-OH-TCC**	**3'-OH-TCC**	**3'-Cl-TCC**	**TCS**	**MeTCS**
1	0.32	0.05	**0.02**	0.15	NA	0.08	0.35	**0.00**
2	0.58	0.13	**0.03**	0.75	0.97	0.74	0.71	0.13
3	0.28	0.35	0.38	0.15	0.17	0.18	0.61	**0.00**
4	0.50	0.05	**0.03**	0.78	0.05	0.57	0.92	**0.00**
5	0.14	0.07	**0.02**	0.61	0.35	0.20	0.47	0.69
6	0.11	**0.04**	0.21	0.43	0.05	0.31	0.40	**0.00**
7	0.67	0.28	0.11	**0.02**	0.28	0.55	0.38	**0.01**
8	0.20	**0.04**	**0.02**	0.33	**0.02**	0.72	0.15	**0.00**
9	0.71	0.40	0.09	0.68	0.62	0.60	0.89	0.22
10	0.19	0.45	**0.00**	0.19	**0.04**	**0.00**	0.59	**0.00**
11	0.43	0.80	0.06	0.14	**0.03**	0.07	0.28	**0.01**
12	0.11	**0.02**	**0.01**	**0.01**	**0.01**	0.80	0.55	**0.00**
13	0.06	**0.02**	**0.01**	**0.03**	**0.01**	0.06	0.49	**0.03**
14	0.19	**0.00**	**0.00**	0.14	**0.02**	0.75	0.07	0.08

15	0.36	**0.01**	0.06	0.26	0.84	0.54	0.73	**0.03**
16	0.12	**0.01**	**0.00**	0.06	0.70	0.80	0.12	**0.00**
17	**0.02**	0.59	**0.00**	**0.01**	0.05	**0.01**	0.25	**0.00**
18	0.44	**0.02**	**0.00**	**0.04**	0.34	0.37	0.92	0.27
19	0.08	**0.01**	**0.00**	**0.04**	**0.03**	0.94	0.64	**0.02**
20	**0.03**	0.37	**0.05**	0.40	0.46	0.17	0.51	**0.01**
21	0.76	**0.01**	**0.02**	0.56	0.29	0.71	0.47	0.34
22	0.77	**0.00**	**0.00**	0.49	**0.02**	0.21	**0.04**	0.27
# <0.05	2	11	16	6	8	2	1	15
# <0.01	1	2	7	0	0	1	0	9

NA = Not applicable.

Table S7. The effect on molar TCC removal of sludge treatment processes at four different sites. Removal is expressed as the molar ratio of all carbanilide congeners to the TCC.

Site #	Assessed Treatment	Molar TCC Removal (%)		
		Before	After	Net Difference
6	Sludge Dewatering	10.3 ± 0.2	11.6 ± 0.6	+1.3
14	Heat Treatment	29.8 ± 3.0	35.0 ± 1.5	+5.2
15	Anaerobic Digestion	1.2 ± 0.01	1.6 ± 0.1	+0.4
16	Anaerobic Digestion	1.1 ± 0.2	3.2 ± 0.1	+2.1